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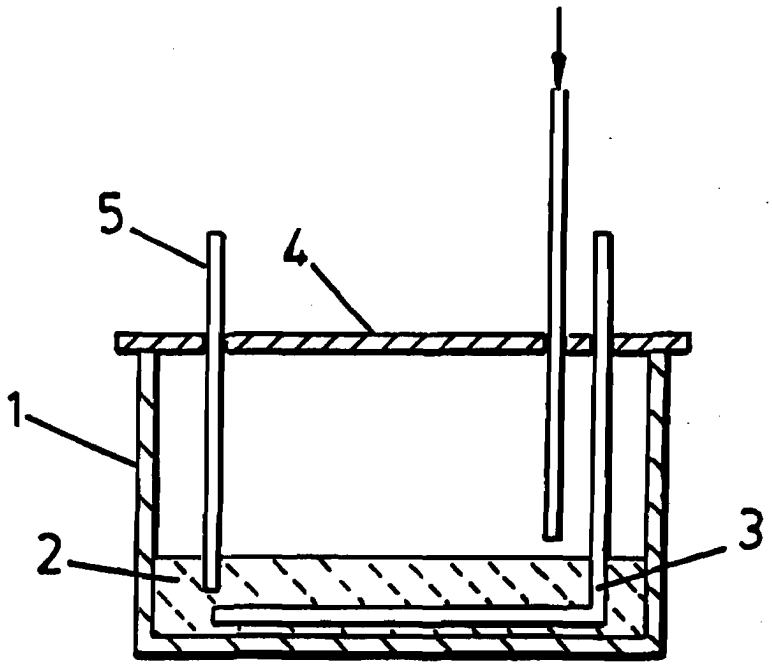
(57) Abstract

The apparatus comprises a container (1) into which culture medium (2) has been introduced. Located within the container and partially submerged by the medium is a main electrode (3), the surface of which is formed by a thin film of gold. This electrically conductive surface supports adherent, variable cells previously grown to near confluence. The container is closed by a lid (4) which is penetrated by a tube (5) filled with an electrochemically conducting medium that is in electrochemical contact with a reference electrode. The end of the tube (5) is immersed in the culture medium. A further tube is provided through which test factors such as stimulants or suppressants can be injected into the container.



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ELECTROCHEMICAL ASSESSMENT OF CELL BEHAVIOUR AND METABOLIC ACTIVITY

The present invention relates to a method and apparatus for the monitoring of electrochemical signals which reflect the metabolic and behavioural activities of living cells.

All cells utilise metabolic pathways based on chemical processes, for example, the oxidation-reduction reactions of energy metabolism. In addition there are hundreds of other chemical processes, especially the electrochemical reactions and ionic pumps' that cross the cell surface, all of which provide the molecular basis for the cell's behavioural activities.

It is well known that cells are electrochemically active, and that changes in energy metabolism often relate to modified cell behaviour. For example, the article "The Cytosensor Microphysiometer: Biological Applications of Silicon Technology" by H M McConnell, J C Owicki, J W Parce, D L Miller, G T Baxter, H G Wada and S Pitchford in the Journal "Science", Volume 257, 25 September 1992 describes a device which is capable of measuring the rate of proton excretion by cells in response to chemical/ligand stimulation. The Cytosensor device is based upon the fact that some cells acidify their surrounding environment because of acidic products of energy metabolism, especially glycolysis. The device measures changes in pH (acidification) that occur as cells release acidic metabolites into their immediate environment, this being related to rates of energy metabolism. The Cytosensor may be used for two

functions, that is as a detector for specific molecules (e.g. ligands) whereby responsive, viable cells in the instrument serve as detectors and amplifiers, and for the investigation of cell function and biochemistry. Thus the Cytosensor is a useful device for monitoring extra cellular pH which generally relates to changes in the energy metabolism of cells. However, there are many other electrochemical processes that cross the cell surface, some of which may contribute to the rate of acidification of the surrounding environment, but many others of which will not. Accordingly, it may be that in some circumstances a significant change in electrochemical activity within a cell does not result in significant change in the rate of acidification of the cell's environment.

The present invention provides a different method and device for assessing cell behaviour and metabolic activity. The present invention is based upon the realisation that the many chemical processes taking place at the cell surface produce electrochemical signals. The "signals" include, for example, potential noise and current noise, the term "noise" being used to indicate the sum of those signals resulting from cellular electrochemical activity. Techniques developed to assess the progress of electrical or electrochemical processes can be applied to the analysis of signals generated by the electrochemical activity of cells.

The present invention provides a method for assessing the metabolic and behavioural activities of cells, wherein electrochemical signals generated by the cells are detected and analysed to provide data representative of cell behaviour.

The term "behavioural activities" is used herein to include ionic transport and all those biological activities recognised as metabolic, proliferative, and locomotory, and cellular responses to exogenous factors.

The invention also provides an apparatus for assessing the metalolic and behavioural activities of cells, comprising a means for the detection of electrochemical signals generated by the cells, and means for analysing the detected signals to provide data reflecting cell behaviour and changes therein.

Cells maintained in vitro under defined culture conditions produce a characteristic electrochemical signal, this being modified upon exposure to one or more chemicals/ligands/biological factors know to affect cell activity. The electrochemical signals generated by cells with or without stimulation, upon analysis, provides a different set of data representative of the behavioural characteristics of those cells. Thus it is believed that a high degree of discrimination is afforded by analysis of the electrochemical processes which reflect, and relate, to modified cell behaviour.

The signals monitored and subsequently analysed, could be, for example, the electrochemical potential noise. Alternatively, fluctuations in electrochemical current may be monitored either separately, or in combination with the electrochemical potential.

The apparatus in accordance with the invention may comprise a container for receiving a culture medium containing a sample of cells to be assessed, the cells being supported by a chemically inert, electronically conductive surface arranged within the

container. An electrochemical pathway in the form of a conducting medium within, for example, a tube and connected to a reference electrode may be arranged within the container in contact with the culture medium. The electrochemical noise detecting means is connected to the conductive surface and the reference electrode to detect electrochemical noise signals generated in the sample. The chemically inert conductive surface may be in the form of a film of gold formed, for example, as a foil or as a deposit on an electrically insulating substrate.

Embodiments of the present invention will now be described, by way of example, with reference to the accompanying drawings, in which:-

Figure 1 is a schematic representation of an apparatus in accordance with the present invention;

Figure 2 is a schematic representation of an electrical circuit for analysing potential noise signals generated in the apparatus of Figure 1;

Figures 3a and 3b are plots of potential 'noise' against time generated by carcinoma cells and fibroblasts, respectively, using the apparatus of Figure 1 and the circuit of Figure 2;

Figure 4a is representation of a power spectrum associated with carcinoma cells produced on the basis of the information shown in Figure 3a and subsequently analysed;

Figure 4b illustrates differences in the power spectra of the two cell types, that is breast carcinoma and fibroblasts;

Figure 5 is a schematic representation of circuitry which may be used to monitor electrochemical current noise signals generated using a modified form of the apparatus shown in Figure 1; and

Figure 6 illustrates a miniaturised probe structure suitable for use in accordance with the invention.

Referring to Figure 1, the illustrated apparatus comprises a container 1 into which culture medium 2 has been introduced. Located within the container and partially submerged by the medium is a main electrode 3, the surface of which is formed by a thin film of gold. This electrically conductive surface supports adherent, variable cells previously grown to near confluence. The container is closed by a lid 4 which is penetrated by a tube 5 filled with an electrochemically conducting medium that is in electrochemical contact with a reference electrode. The end of the tube 5 is immersed in the culture medium. A further tube is provided through which test factors such as stimulants or suppressants can be injected into the container.

Referring now to Figure 2, reference numerals 1, 3, 4 and 5 are used for the same components as are identified by those numbers in Figure 1. A digital voltmeter 6 monitors the potential between electrodes 3 and 5 and the output of the digital voltmeter is applied to PC 7. The PC processes the voltmeter output to generate a signal which drives a display 8 and a hard copy printer 9. In addition, the PC processes the data to provide an output to a spectrum analyser 10.

Figures 3a and 3b show results obtained using the apparatus illustrated in Figures 1 and 2, the results being represented by the rest potentials monitored against

time for breast carcinoma cells (Figure 3a) and human fibroblasts (Figures 3b). To further illustrate the differences which arise with different cells, the following table represents example of mean rest potential against time and standard deviation for the probe surface under different conditions:

Probe surface condition	Mean rest potential /mv	standard deviation in rest potential /mv
Bare (cell-free)	-61.1	11.1
Cancer cell (BC 8701)	-129.6	10.7
Fibroblast (HAC90 P2)	-95.6	5.2

Figure 4a illustrates the power spectrum derived from analysis of the signals from the unstimulated breast carcinoma cells. Figure 4b represents the power spectra differences observed for separate cultures of breast carcinoma cells and normal fibroblasts, to illustrate that different types of cell generate different electrochemical signals. Thus Figure 4b represents the difference between the results shown in Figure 4a for carcinoma cells and the equivalent results (not shown) for fibroblasts. In the absence of differences, the profile of Figure 4b would approximate zero across the frequency range.

As an alternative to monitoring fluctuations in electrochemical potential, it is possible to monitor fluctuations in electrochemical current. Figure 5 schematically illustrates on arrangement for monitoring electrochemical current noise. As shown in Figure 5, a third electrode 13 is introduced, this being partially immersed in the

culture medium in exactly the same manner as electrode 5. Electrodes 5 and 13 are connected to an electrochemical potential noise monitoring apparatus 14 which provides on output 15 output signal representative of Vn, that is the rms or standard deviation of the potential noise signal. A zero resistance ammeter 16 is connected across electrodes 3 and 13 and produces on output 17 an output signal corresponding to Im, that is the dc coupling current.

The output 17 is connected to an electrochemical current noise measuring apparatus 18 which provides on output 19 an output signal corresponding to In, that is the rms or standard deviation of the current noise signal. The outputs 15 and 19 are applied to a circuit 20 for comparing the electrochemical potential noise signal and the electrochemical current noise signal. The circuit 20 provides on output 21 an output signal Rn which represents impedance noise and is equal to Vn/In. The output 21 is effectively indicative of the overall rate of electrochemical activity.

The structure illustrated in Figure 5 provides effectively four outputs, each of which varies in a manner that is indicative of the rate and/or nature of the electrochemical activity of the cells to which the electrodes 3, 5 and 13 are exposed. It is believed that if a comparison is made between the signals appearing on outputs 17 and 19 important information can be gained as to the nature of the cell activity. Accordingly, a comparator circuit 22 is connected to the outputs 17 and 19 and provides on output 23 an output signal which is representative of Im/In.

The output 23 results from the comparison of the meaning coupling current to the standard deviation values of the electrochemical current noise signal, and it is

believed that this will provide information characteristic of the cell activity that generated the signal.

A further comparator 24 is provided to compare the output 21 with the output 23 to produce a still further output 25 which again is indicative of characteristic features of the electrochemical activity.

The arrangement of Figure 5 enables the generation of a significant number of different signals all based on the same electrochemical activity. It is believed that different cell conditions which might result in, for example, similar fluctuations in electrochemical potential are unlikely to result in close similarity between the other available outputs. Thus the arrangement of Figure 5, improves the probability of being able to adequately discriminate between different cell behavioural patterns.

The apparatus illustrated in Figures 2 and 5 is very similar to that illustrated in European Patent Specification Nos. 0084404 and 0302073 respectively. Those patent specifications are concerned with the monitoring of corrosion but it is believed that the techniques developed for monitoring corrosion by reference to electrochemical noise are equally applicable to the analysis of electrochemical activity produced by cells. It will be appreciated that although Figures 2 and 5 show examples of possible apparatus for monitoring electrochemical signals, there are alternative arrangements available as will be appreciated by reference to the numerous academic and patent publications relating to for example the analysis of electrochemical noise generated within corroding materials. Further research is expected to enable specific cell and/or tissue behavioural activities to be correlated with associated and characteristic

electrochemical signals. It is believed, however, that since all cellular biological activities are based on chemical processes and electrochemical reactions, the analysis of electrochemical signals generated by cells will give access to a large amount of data some of which relate to specific behavioural activities. For example, since stimulated or cancer cells invariably have a more active behaviour, such as elevated proliferative activity, it is very likely that the underlying chemical reactions taking place within such cells are different from those taking place in normal cells which provide more consistent functional activities. Thus each specific cell type is likely to have a unique "signature" of cell behaviour reflecting complex combinations of electrochemical reactions and those "signatures" should be accessible to an analysis of associated electrochemical signals.

In the experiments, illustrative results of which are represented in Figures 3 and 4, and Table 1, both the voltage responses and the associated power spectra for the metabolically active cancer cell culture and fibroblast culture were measurably different.

In order to improve the amount of data which can be obtained from a limited number of cells, and to enable the improvement of procedures, it is desirable to provide a miniaturised apparatus which is capable of providing more than one output for a single body of cells, and which is capable of providing control outputs representing background signals which may arise even in the absence of cells. Figure 6 schematically illustrates one structure which can provide such capabilities.

The probe assembly of Figure 6 comprises six metallic wires 26, which may be gold, embedded in an electrically insulating body 27 of for example cold curing or hot setting resin. The end surface of each of the wires is exposed so as to define an electrode surface onto which cells may be deposited. The surface of the body 27 on which the ends of the wires 26 are exposed may be polished using a lapidary wheel. Each of the six wires 26 is separately connected by a respective conductor 28 to a respective monitoring circuit (not shown). Thus independent outputs are provided for each of the six wires.

The wires 26 are arranged in two groups of three located on opposite sides of a notional line 29. The exposed ends of one of the groups (to the left of line 29 in Figure 6) and the adjacent surface of the body 27 are placed in contact with the body of cells to be investigated. Some of the cells will become attached to the ends of the wires. such cells being represented by small circular areas in Figure 6. The other group of wires 26 is cell free. Cells could be deposited in this manner by for example covering one group of wires and the adjacent body surface with an insulating layer of film. e.g. Parafilm, during the cell attachment process, and removing the film prior to monitoring cell activity.

Figure 6 shows six wires 26 in total, but it will be appreciated that both the number and diameter of the wires may be adjusted to suite particular applications. Wire diameters of from 0.2 to 2.0mm will be suitable in some circumstances.

The apparatus of Figure 6 permits the monitoring of replicate electrodes. with and without cells attached. in a single culture apparatus. This provides improved

consistency of data, convenience and simplicity compared to the use of separate culture units.

The investigation so far has been concerned with in vitro studies. It is believed, however, that the present invention could be adapted and developed for use in vivo.

There are many potential applications for the present invention. For example, the invention enables the provision of an analytical tool for the study of specific cell behaviour in vitro, such as the effects of pharmaceutical compounds, hormones, cytokines, prostaglandins, mutagens etc on selected target cells; biocompatibility screening, e.g. in vitro evaluation of prosthetic material surfaces for improvement of implant osseointegration; the identification of specific cell types in vivo, for example, detecting the presence of certain tumour cells and their location within specific tissues; the selection and optimisation of anti-cancer treatment in ex-vivo culture; and an assessment of brain activity and regional variations of such activity.

It will be appreciated that the present invention would for example, enable data to be captured in real time. Accordingly, the normally slow progress of investigations related to the effects of, for example, a particular chemical or factor on cell activity, can be transformed by the application of the present invention. Furthermore, given that the invention relies upon electrochemical signals generated by cells in vitro, ex vivo or in vivo, it is believed possible to identify particular cell types without inducing changes or modifications in their behaviour, that is without it being

essential to stimulate or suppress specific cell activities. Further information can be obtained subsequently by stimulation or suppression of the same cells.

CLAIMS

1. A method for assessing the metabolic and behavioural activities of cells, wherein electrochemical signals generated by the cells are detected and analysed to provide data representative of cell behaviour.

2. A method according to claim 1, wherein the cells are stimulated or suppressed by exposing them to one or more exogenous factors which may affect cell activity, and the electrochemical response to those stimulating or suppressive factors is monitored by reference to the detected electrochemical signals.

3. A method according to claim 1 or 2, wherein fluctuations in the electrochemical potential at the cell surface are detected and analysed.

4. A method according to claims 1, 2 or 3, wherein fluctuations in the electrochemical current at the cell surface are detected and analysed.

5. An apparatus for assessing the metabolic and behavioural activities of cells, comprising means for detecting electrochemical signals generated by the cells, and means for analysing the detected signals to provide data representative of cell behaviour.

6. An apparatus according to claim 5, comprising a container for receiving a culture medium of a sample of cells to be assessed, a chemically inert electrically conductive surface arranged within the container onto which the cells may be grown or attached, and a reference electrode arranged within the container to contact the culture medium, the detecting means being connected to the conductive

surface and the reference electrode to detect electrochemical signals generated in the sample.

7. An apparatus according to claim 6, wherein the conductive surface is in the form of a conductive film formed on an electrically insulating substrate.

8. An apparatus according to claim 6, wherein the conductive surface is in the form of a wire extending through and to the surface of an insulating body.

9. An apparatus according to claim 8 comprising a plurality of wires each connected to a respective detecting means.

10. An apparatus according to any one of claims 5 to 9, wherein the detecting means detects fluctuations in the electrochemical potential.

11. An apparatus according to any one of claims 5 to 10 where in the detecting means detects fluctuations in the electrochemical current.

12. A method for assessing the behavioural activity of cells by reference to electrochemical signals generated by the cells. substantially as hereinbefore described with reference to the accompanying drawings.

13. An apparatus for assessing the behavioural activity of cells by reference to electrochemical signals generated by the cells, substantially as hereinbefore described with reference to the accompanying drawings.

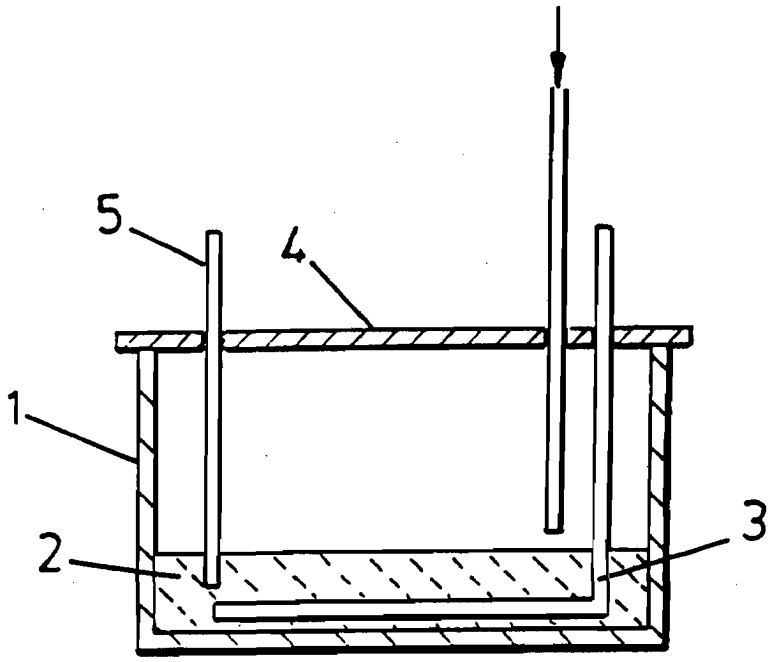

FIG. 1

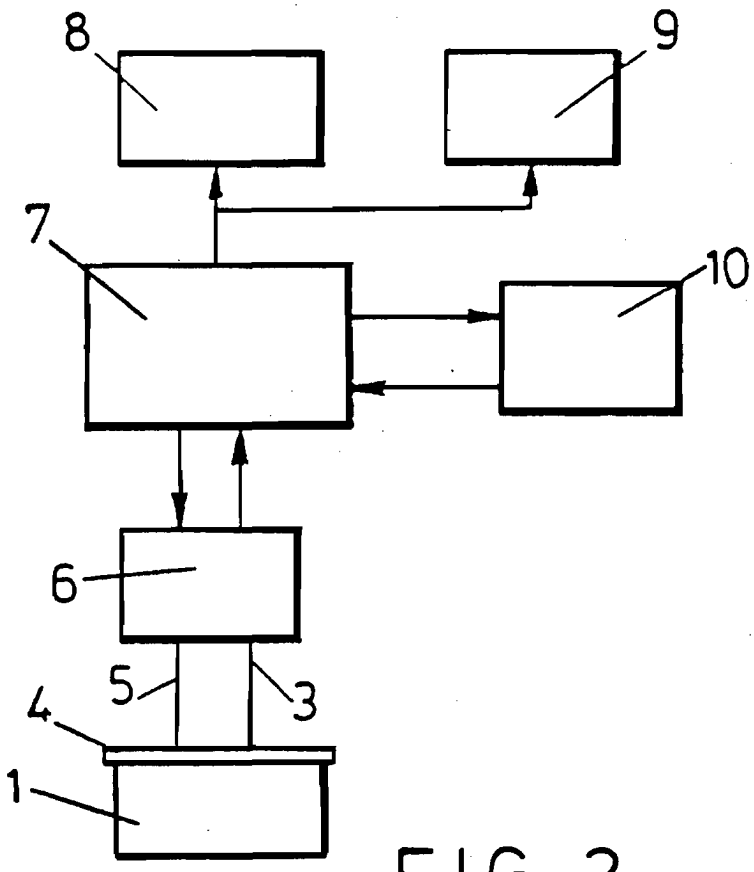

FIG. 2


-0 1138
-0 1139
-0 1140
-0 1141
Volts
Time

FIG. 3a

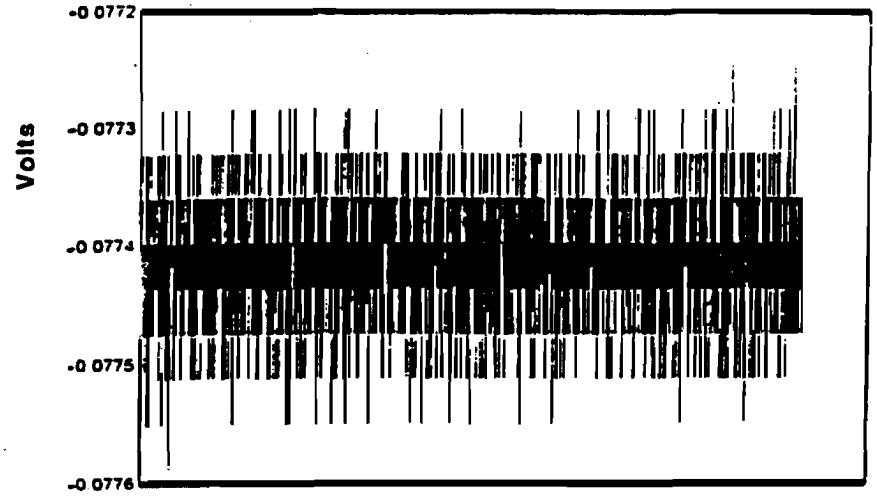
-0 0772
-0 0773
-0 0774
-0 0775
-0 0776
Volts
Time

FIG. 3b

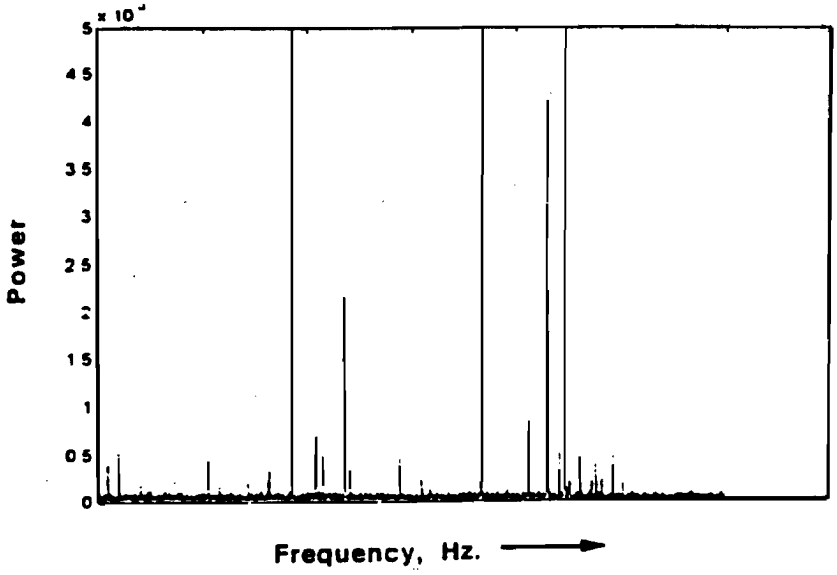
x 10⁻⁴
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Power
Frequency, Hz.

FIG. 4a

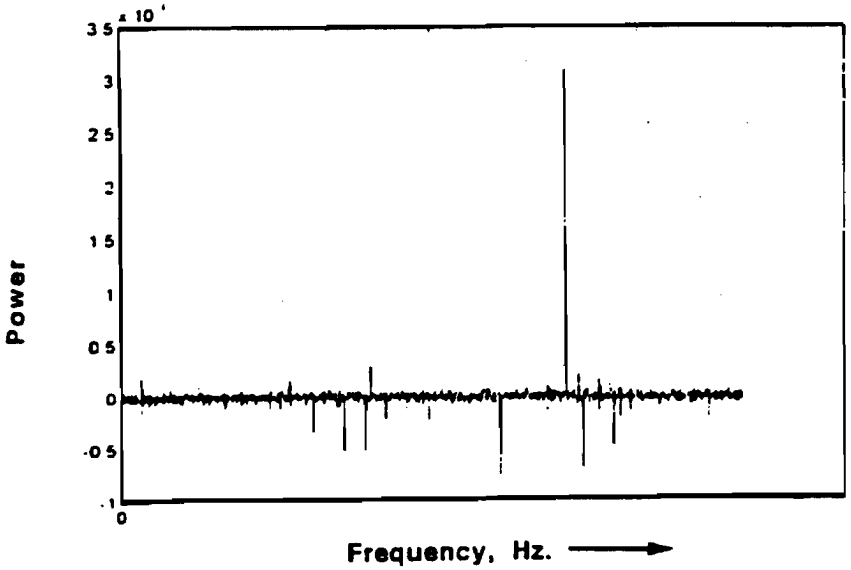
x 10⁻⁴
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
0
Power
Frequency, Hz.

FIG. 4b

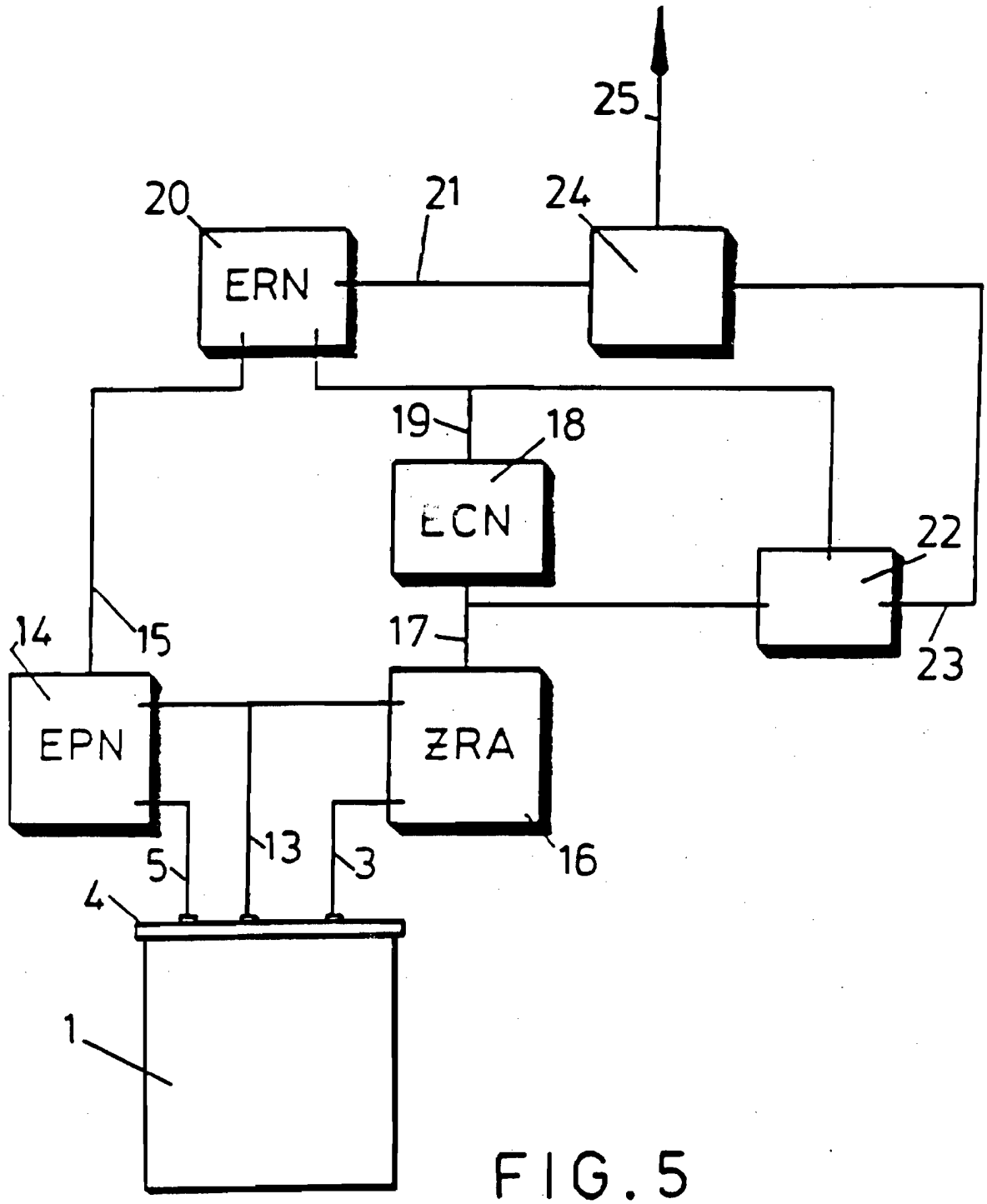

FIG. 5

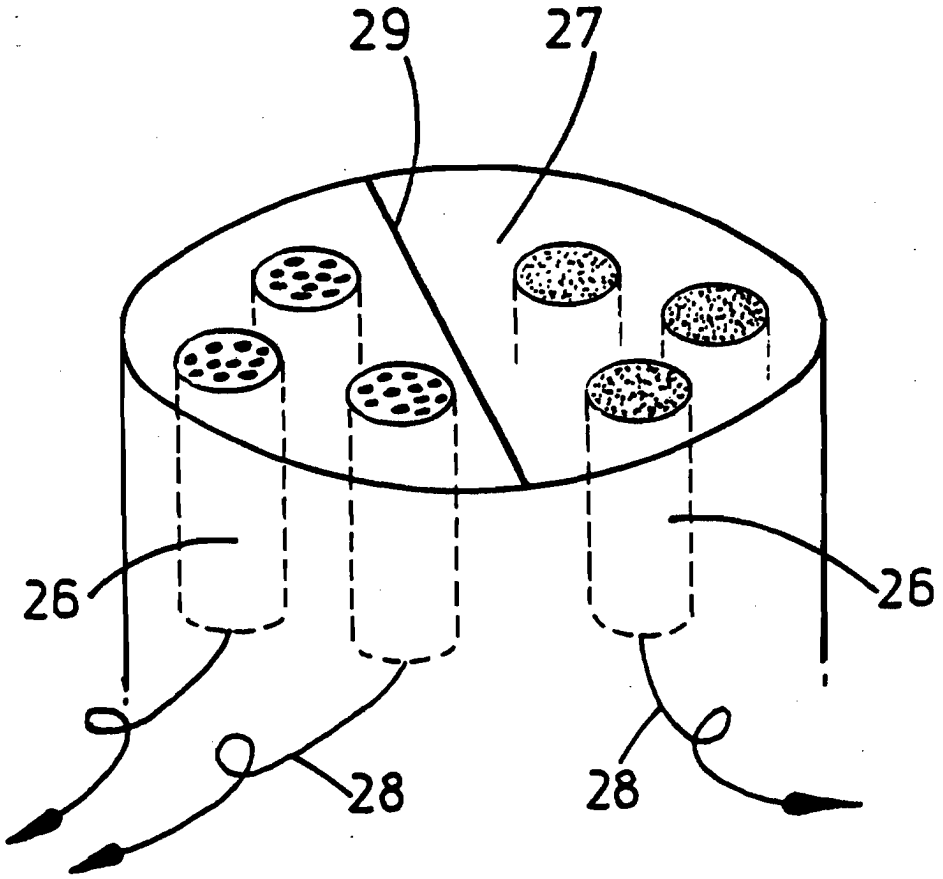

FIG.6

A. CLASSIFICATION OF SUBJECT MATTER

G 01 N 27/416,C 12 M 1/00

According to International Patent Classification (IPC) or to both national classification and IPC 6

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G 01 N,C 12 M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	EP, A, 0 627 661 (AVL) 07 December 1994 (07.12.94), the whole document. --	1-7, 10,11
Y	EP, A, 0 028 793 (KYOWA HAKKO KOGYO) 20 May 1981 (20.05.81), the whole document. --	1-7, 10,11
Y	SCIENCE, vol. 257, issued 1992, September 25 H.M. McCONNEL et al. "The Cytosensor Microphysiometer: Biological Applications of Silicon Technology", pages 1906-1912, especially page 1906, column 1, line 1 -	1-7, 10,11

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[ ] Patent family members are listed in annex.

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Date of the actual completion of the international search

19 December 1995

Date of mailing of the international search report

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International Application No PCT/GB 95/02297

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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	page 1909, column 1, line 7 (cited in the application). ----	

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif à la demande de brevet international n°

PCT/GB 95/02297 SAE 118896

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EP A1 627661	07-12-94	JP A2 6335653	06-12-94
EP A2 28793	20-05-81	DE C0 3065478	08-12-83
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		EP B1 28793	02-11-83
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